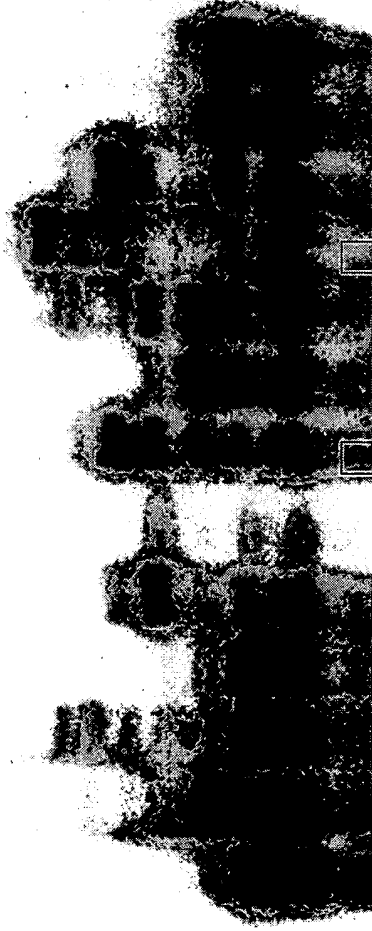
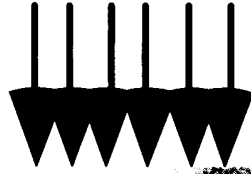




Incorporated (5' to 3')
GATC AGAAAG

5 base extension
 4 base extension
 3 base extension
 2 base extension
 1 base extension
 19 base "TOP"



dNTP - G A G * A * A G A A

LANE 1 2 3 4 5 6 7 8 9 10 11 12 13

implies presence of an ANS tag attached via
 the dNTP γ -phosphate

FIG. 1

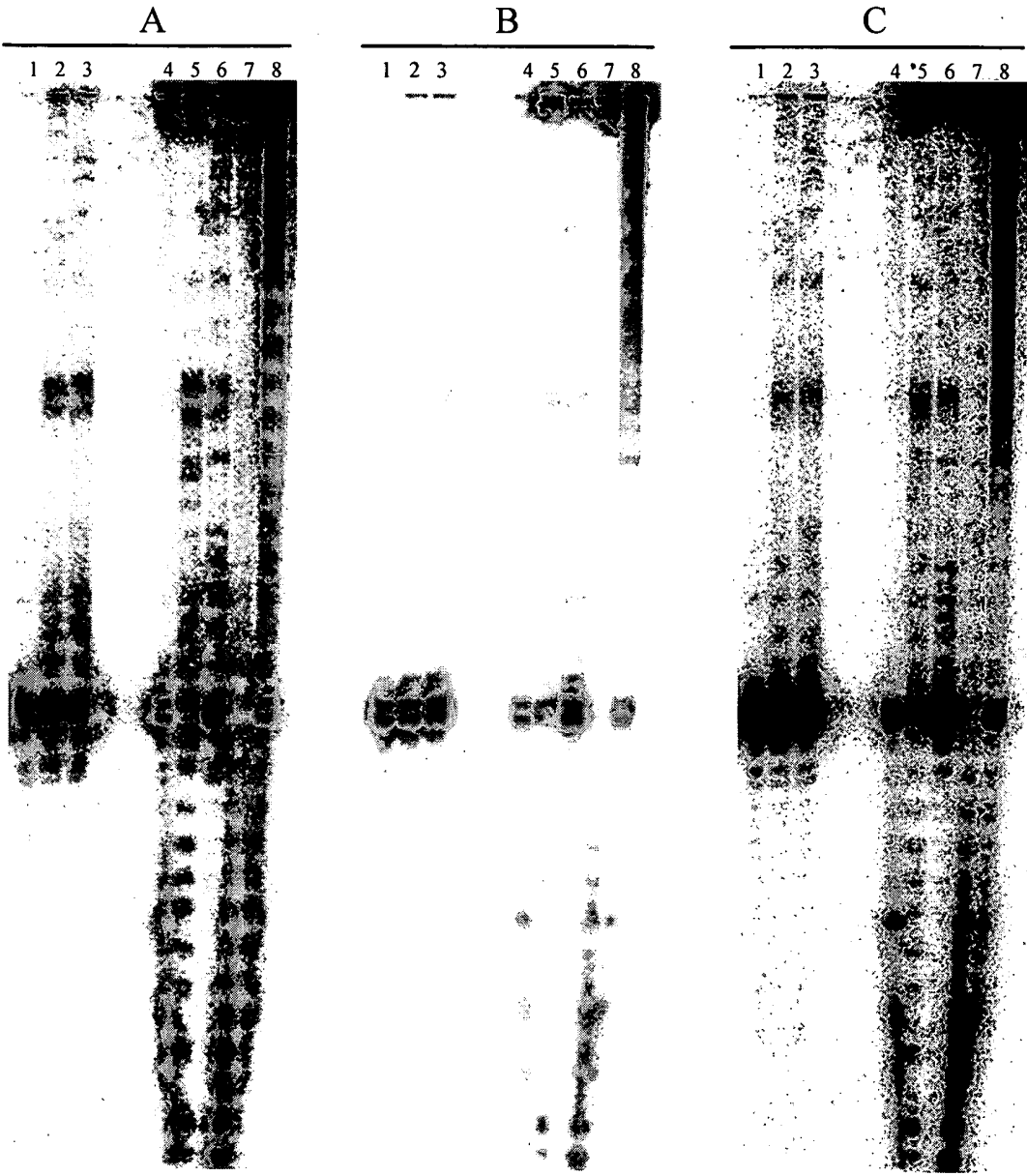
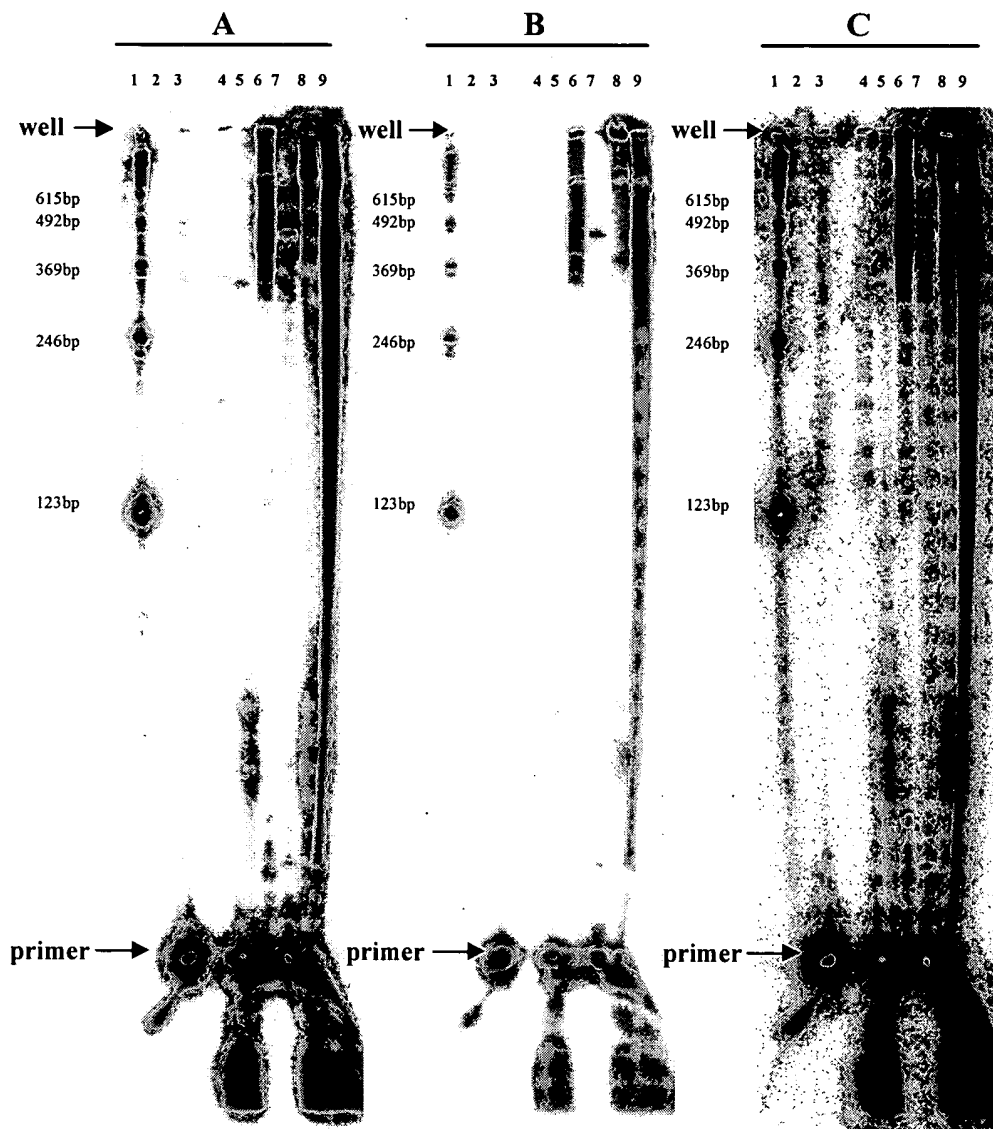
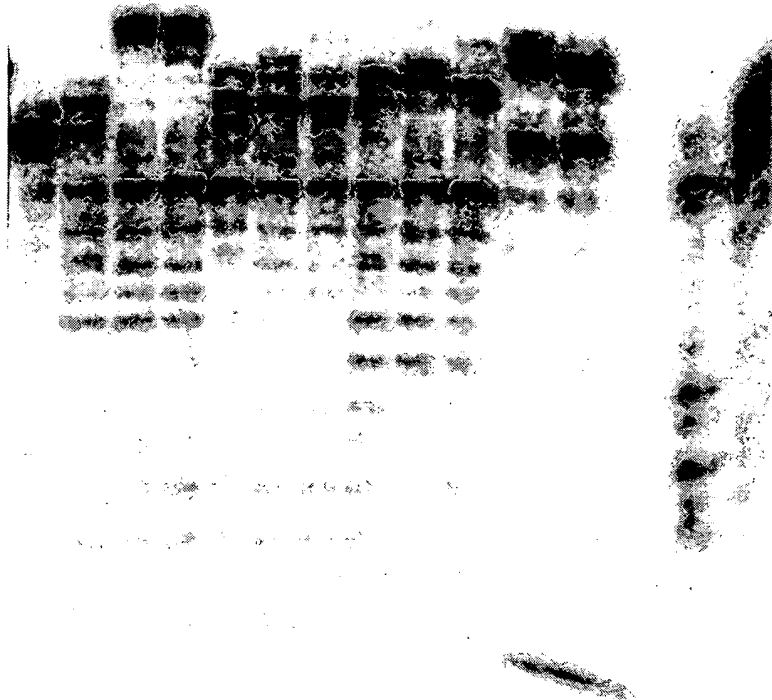


FIG. 2

**FIG. 3**

| | | <u>Klenow</u> | | | | | | | | | <u>Taq</u> | |
|--------------|---|----------------|----|------------|---------------|----|------------|----------------|----|------------|----------------|------------|
| Enzyme | - | + | + | + | + | + | + | + | + | + | + | + |
| Primer (TOP) | + | + | + | + | + | + | + | + | + | + | + | + |
| Template | - | <u>BOT-3TC</u> | | | <u>BOT-TC</u> | | | <u>BOT-Sau</u> | | | <u>BOT-3TC</u> | |
| Nucleotide | - | dG | dA | γ A | dG | dA | γ A | dG | dA | γ A | dA | γ A |



γ implies the presence of an ANS-tag attached at the dNTP
 γ -phosphate

FIG. 4

| | <u>Pfu</u> | | | | | | | | | | <u>Taq</u> | | | |
|--------------|------------|----|------------|----------------|---------------|----------------|----------------|----|----|------------|------------|----|------------|----|
| Enzyme | - | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Primer (TOP) | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Template | - | - | - | <u>BOT-3TC</u> | <u>BOT-TC</u> | <u>BOT-Sau</u> | <u>BOT-3TC</u> | | | | | | | |
| Nucleotide | - | dA | γ A | - | dG | dA | γ A | dG | dA | γ A | dG | dA | γ A | dA |



γ implies presence of an ANS-tag attached via the
dNTP γ -phosphate

FIG. 5

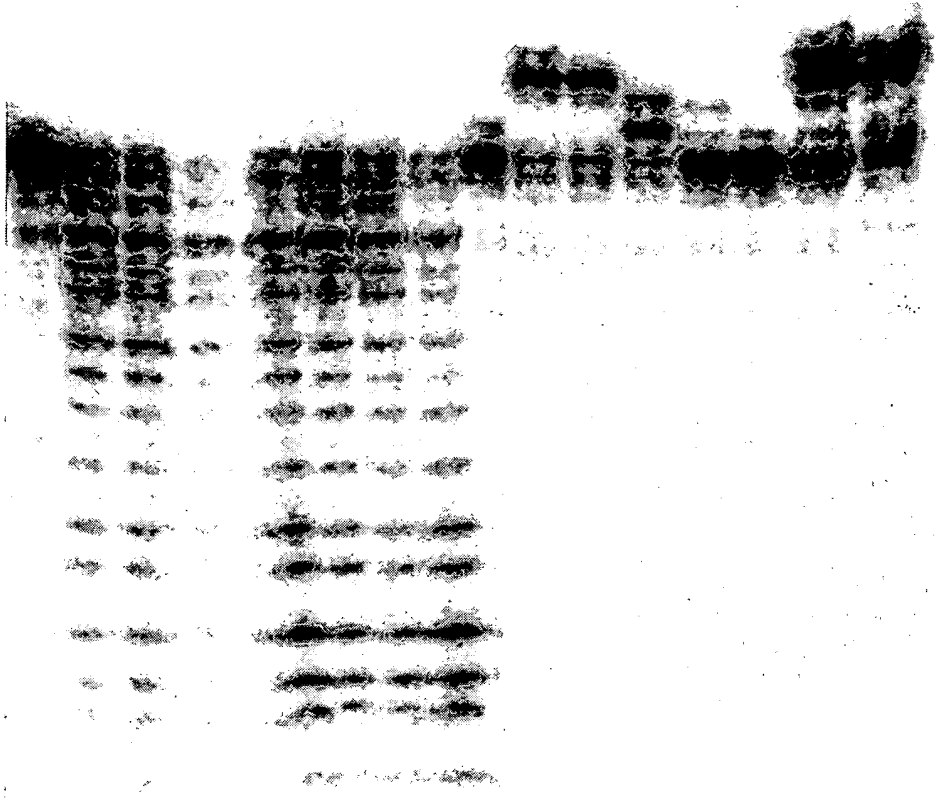
| HIV RT-1 | | | | | | | | | | Taq | |
|--------------|---|---------|----|------------|--------|----|------------|---------|----|------------|---------------|
| Enzyme | - | + | + | + | + | + | + | + | + | + | + |
| Primer (TOP) | + | + | + | + | + | + | + | + | + | + | + |
| Template | | BOT-3TC | | | BOT-TC | | | BOT-Sau | | | BOT-3TC |
| Nucleotide | - | dA | dG | γ A | dA | dG | γ A | dA | dG | γ A | dA γ A |



γ implies presence of an ANS-tag attached via
the dNTP γ -phosphate

FIG. 6

| | <u>T7</u> | | | | | | | | <u>Sequenase</u> | | | | | | | | <u>Taq</u> | |
|--------------|-----------|----------------|----|----|------------|----------------|----|------------|------------------|----------------|------------|----|----|----------------|----|------------|------------|----------------|
| Enzyme | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Primer (TOP) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Template | | <u>BOT-3TC</u> | | | | <u>BOT-Sau</u> | | | | <u>BOT-3TC</u> | | | | <u>BOT-Sau</u> | | | | <u>BOT-3TC</u> |
| Nucleotide | - | dG | dA | dA | γ A | dG | dA | γ A | dG | dA | γ A | dG | dA | γ A | dA | γ A | | |

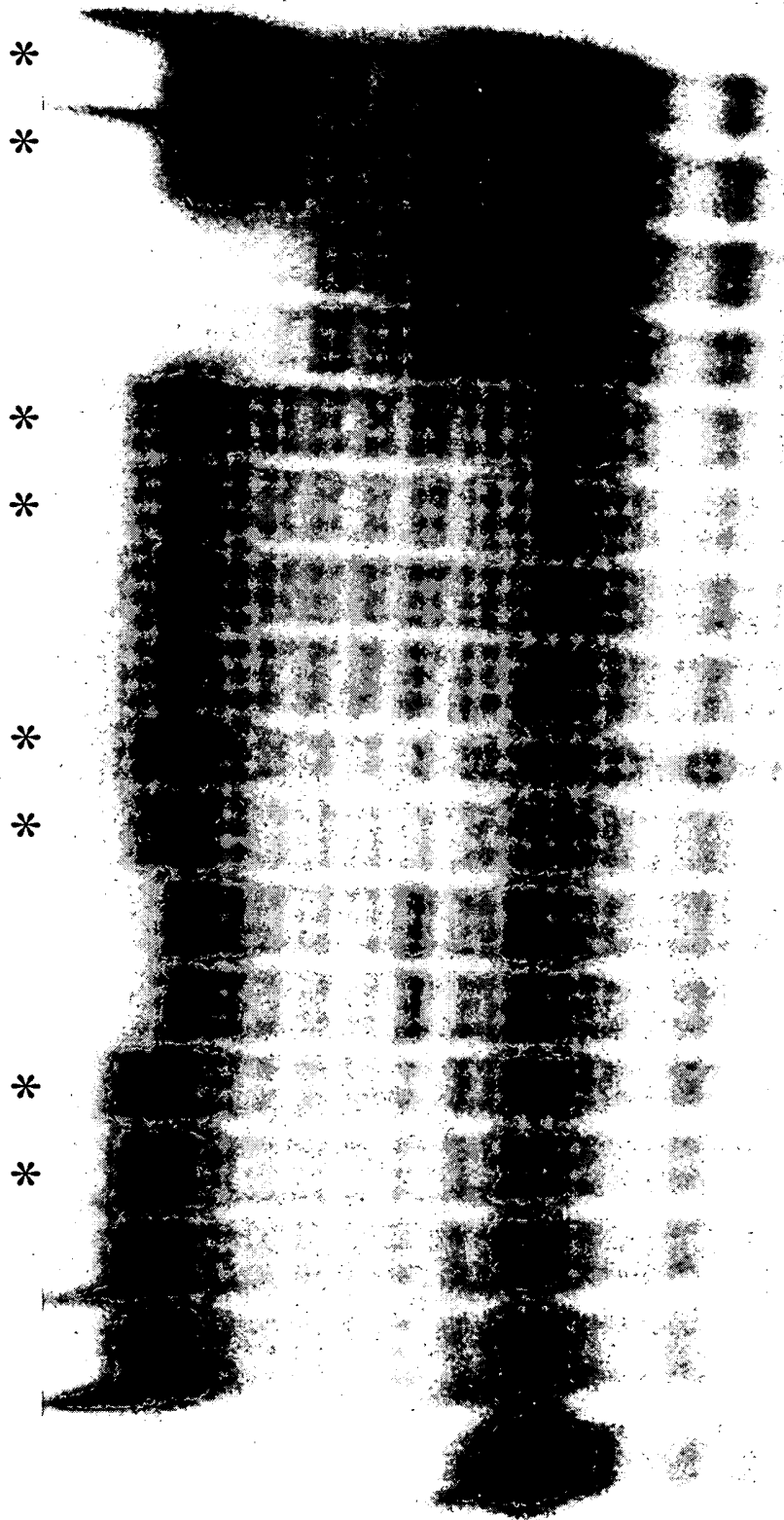


γ implies presence of an ANS-tag attached via
the dNTP γ -phosphate

FIG. 7

* - HEAT TREATED

| dATP | dATP | ANS-dATP | ANS-dATP | TTP | TTP | ANS-TTP | ANS-TTP |
|------|------|----------|----------|-----|-----|---------|---------|
|------|------|----------|----------|-----|-----|---------|---------|



TOP

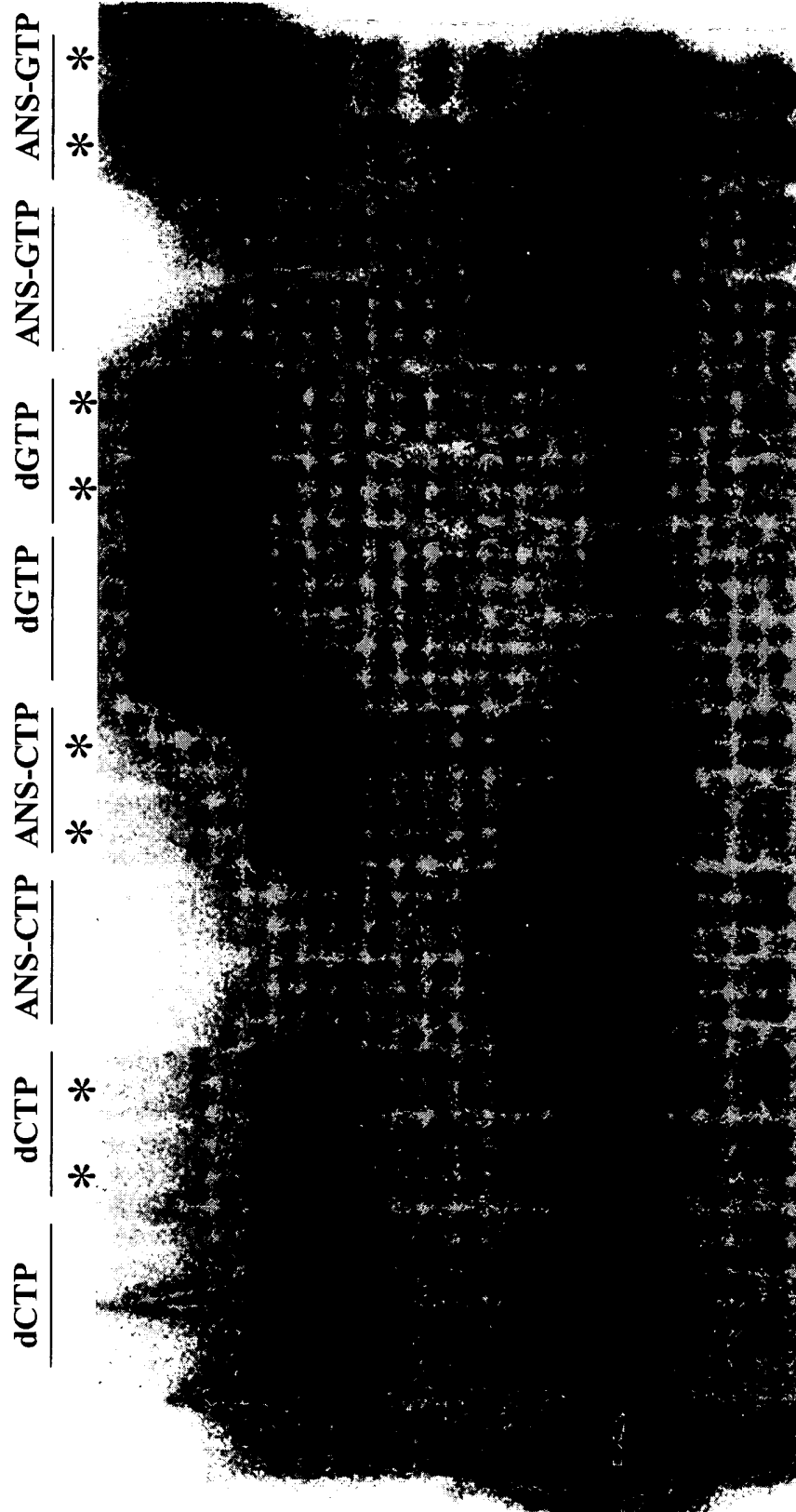
BOT T6T

BOT A6A

10 μ M each dNTP; *Taq* DNA Polymerase; extension 30' @ 37°C

FIG. 8

* - HEAT TREATED



TOP BOT G6G BOT C6C

10 μ M each dNTP; *Taq* DNA Polymerase; extension 30' @ 37°C

FIG. 9

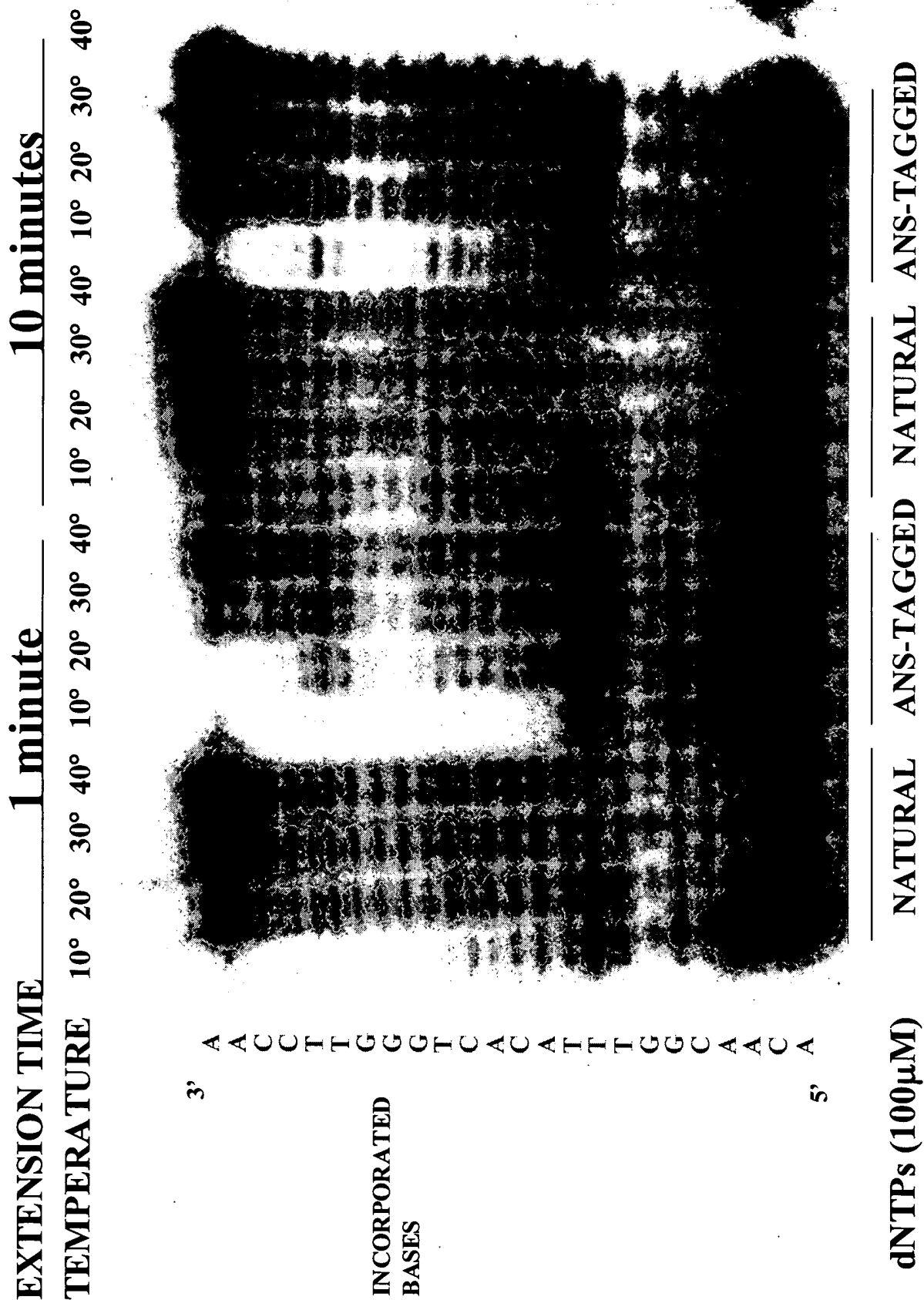


FIG. 10

Primer sequence: 5' GGTAAGCGGCCGCATG 3'

Template sequence: 3' CCATGATTCGCCCGCGTACTGTTGCCAAATGTGACCCAAGTT 5'

1 2 3

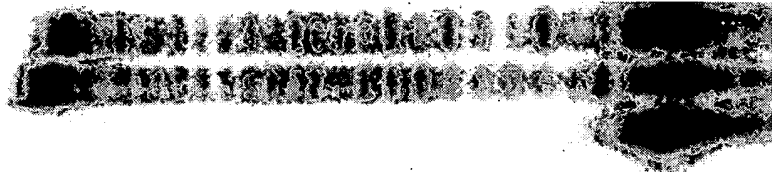
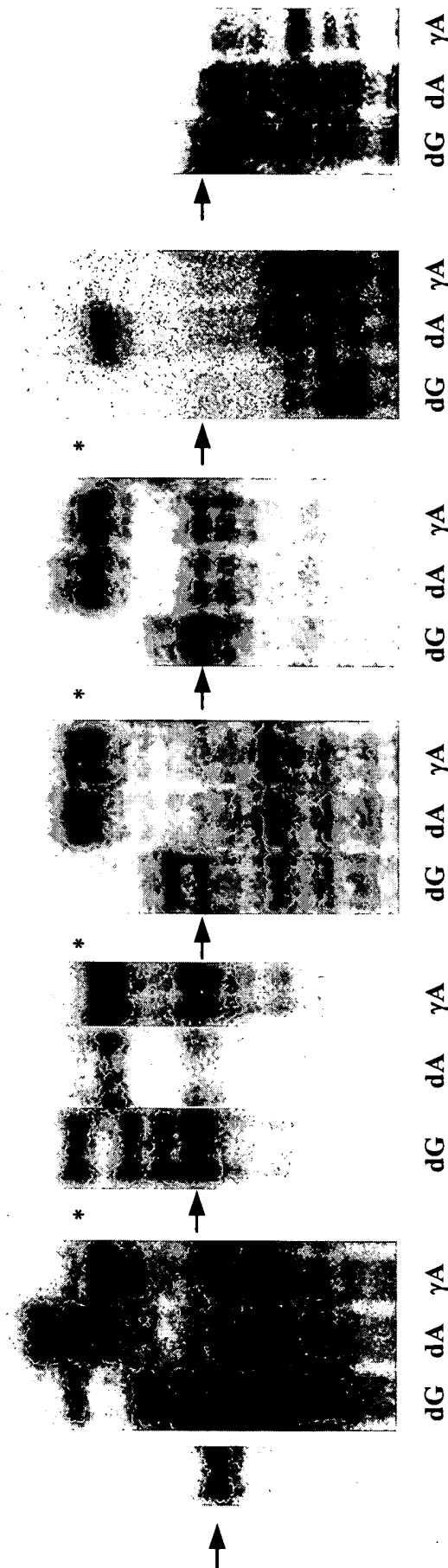


FIG. 11

Primer sequence: 5' GGTACTAAGCGGCCGCATG 3'
 Template sequence: 3' CCATGATTCCGCCGGCTACTTTC5'

POLYMERASE

| | | | | | |
|------------|----------|--------|-----------|------------|----|
| <i>Taq</i> | HIV RT-1 | Klenow | Sequenase | <i>Pfu</i> | T7 |
|------------|----------|--------|-----------|------------|----|



Different Polymerases React Differently to the ANS-g-modified Nucleotides: primer extension reactions were performed to determine the ability of various polymerases to incorporate -tagged dNTPs during DNA polymerization. Control reactions contained natural dNTPs to monitor for template-directed nucleotide incorporation as well as for misincorporation. The reactions were performed in the appropriate buffer and contained the specified polymerase, primer/template duplex (radiolabeled 'TOP' primer annealed to 'BOT-3TC' template), and only the indicated dNTP. The reactions were carried out at room temperature or at 37C for 30 minutes and were stopped by the addition of 0.5mM EDTA. The volume of the reaction was then reduced to approximately 2-4l, loading dye was added and the polymerization products were electrophoresed through a 20% denaturing polyacrylamide gel. Arrows indicate the position of the free labeled 'TOP'. Asterisks indicate 3-base extension.

FIG. 12

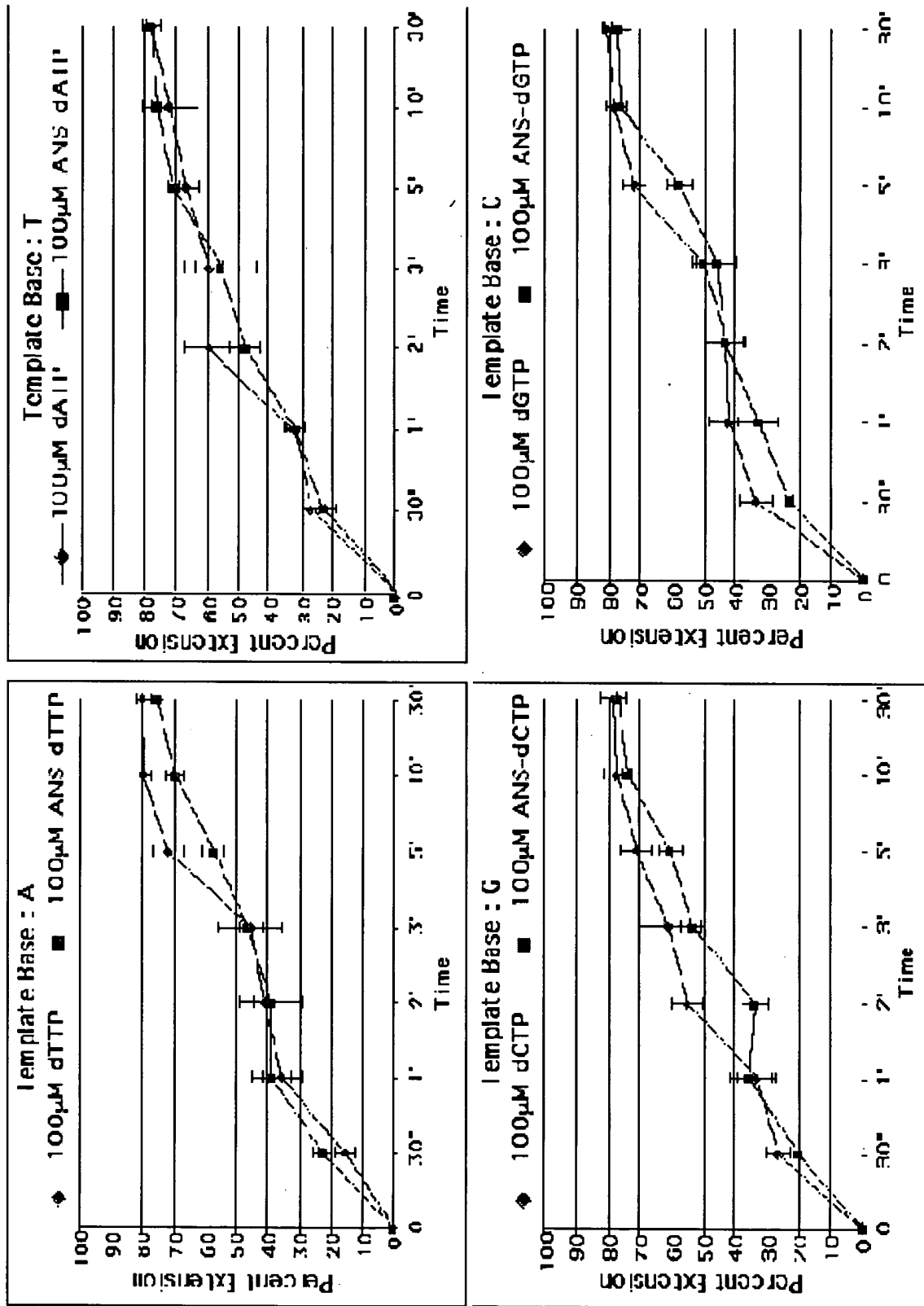
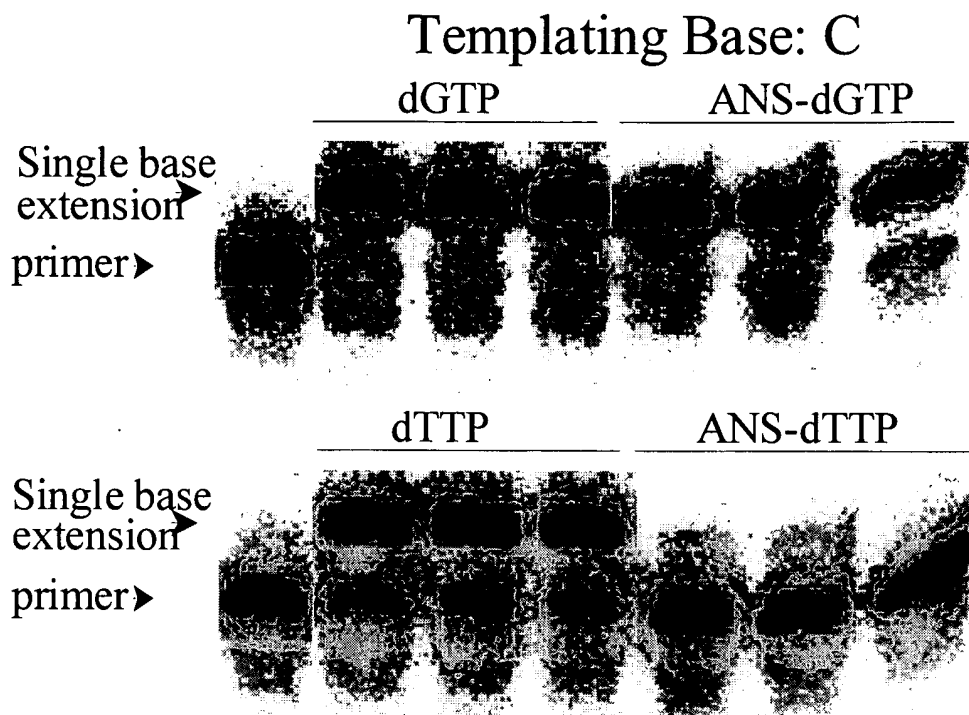
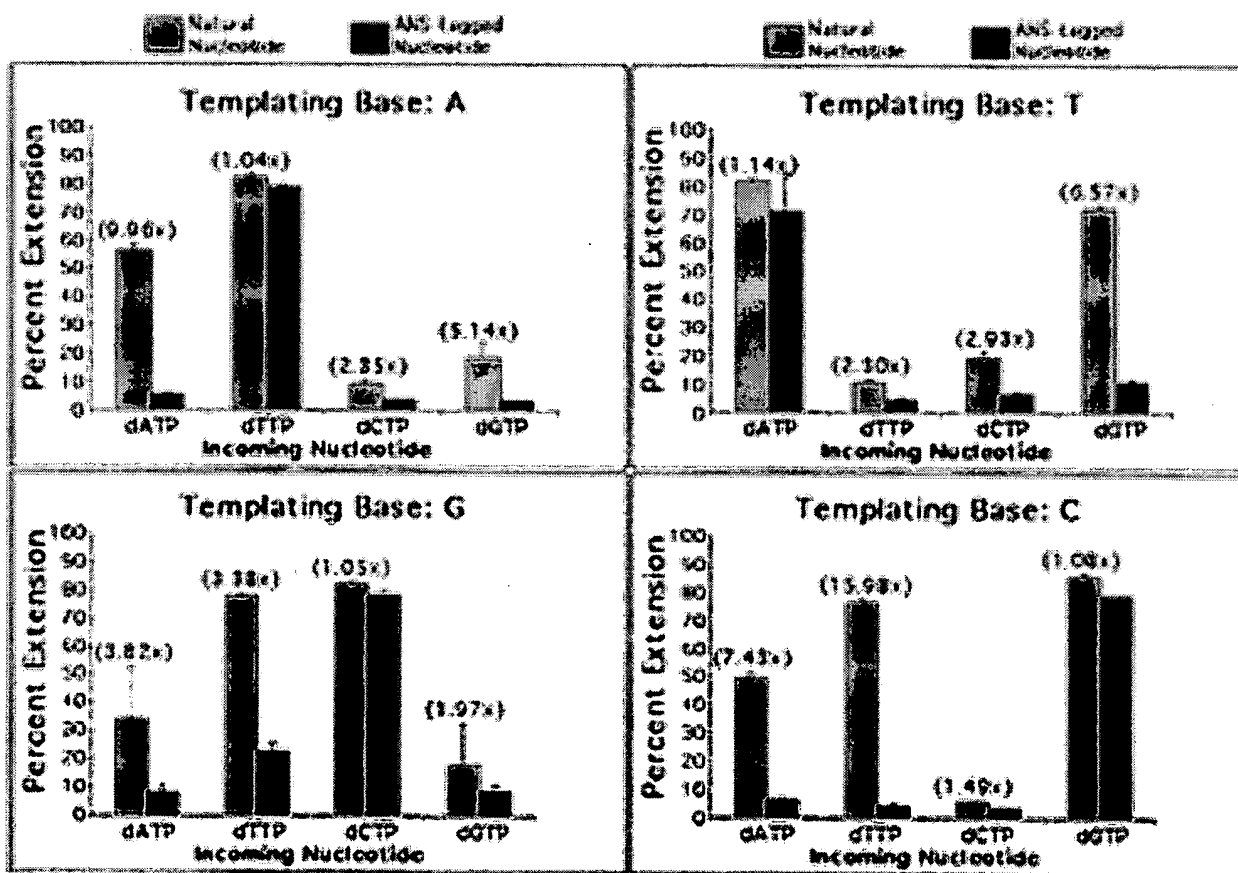


FIG. 13

**FIG. 14 TOP****FIG. 14 BOTTOM**